Novel K5 and K14 Mutations in German Patients with the Weber–Cockayne Variant of Epidermolysis Bullosa Simplex

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We report novel keratin 5 and 14 gene mutations in four unrelated German families with the localized subtype of the dominantly inherited blistering disease epidermolysis bullosa simplex Weber–Cockayne (MIM# 131800). The mutations are located in the keratin 14 L12 linker region (D273G), the keratin 5 L12 linker (M327K and D328H), and the H1 domain of keratin 5 (P156L). These mutations add to those previously reported and provide further evidence of phenotype-genotype correlations in epidermolysis bullosa simplex subtypes. The above mutations in mildly affected patients underline the relevance of the keratin linker regions for the epidermolysis bullosa simplex Weber–Cockayne phenotype and keratin filament integrity. In addition, they confirm that the gene segments encoding the linker regions represent hotspots for mutations. ***Key words: intermediate filaments/keratin genes. J Invest Dermatol 111:900–902, 1998***

Epidermolysis bullosa simplex (EBS) is a group of genetic skin disorders with an autosomal dominant mode of inheritance. The incidence of all types of the disease is ≈1:50,000. The EBS group is characterized by intraepidermal blister formation due to basal keratinocyte lysis. Blisters are induced by minor mechanical stress and appear more frequently in warm and humid weather. Generally, improvement of blistering occurs with advancing age.

According to the severity of symptoms, EBS is subdivided into three major subtypes. The most severe type of EBS is Dowling–Meara, showing generalized herpetiform blistering beginning from birth, accompanied by progressive palmo-plantar hyperkeratosis. Oral mucosal involvement is common. In contrast to the other forms of EBS, EBS-Dowling–Meara skin biopsies are ultrastructurally typified by circumscribed clumps of keratin intermediate filaments (KIF) in basal epidermal keratinocytes. The Koebner (K) type is associated with milder generalized blistering. The mildest and most common form of EBS is Weber–Cockayne (WC), with blistering restricted primarily to the hands and feet (Fine *et al*, 1991); however, suction-induced blisters in apparently unaffected nonacral skin of EBS-WC patients showed the same histologic findings (Taylor *et al*, 1993)

In recent years the molecular basis of EBS began to become apparent by means of molecular genetics and transgenic mouse models. Subsequent sequence analysis of EBS patients revealed that point mutations in the basal keratins K5 and K14 are clustered in distinct regions of the central rod domain, leading to the discovery of different genetic hotspots for each particular disease subtype (for review, see Corden and McLean, 1996; Fuchs, 1996; Joseph and Rothnagel, 1996; Korge and Krieg, 1996). Almost all EBS-Dowling–Meara mutations are located in the highly conserved ends of the α-helical rod. EBS-K mutations are clustered in the more internal parts of the rod domain, in most cases within the 1B or 2B segments of K14. In contrast to EBS-Dowling–Meara and EBS-K, mutations of the EBS-WC type are situated outside the α-helical rod within the L12 linker region of K5 and K14 and the H1 domain of K5 (Chan *et al*, 1993, 1994; Rugg *et al*, 1993; Ehrlich *et al*, 1995; Matsuki *et al*, 1995).

In this report we describe novel mutations in K5 and K14 genes in four unrelated German families with EBS-WC in close vicinity of previously reported mutations, supporting the observation that these regions represent mutational hotspots for EBS-WC.

MATERIALS AND METHODS

Families Subjects to study were four German patients (two familiar and two spontaneous) that showed the typical clinical features of EBS-WC such as onset in early childhood or infancy, blistering restricted to palmoplantar regions, worsening in hot weather, and absence of oral cavity lesions or milia (for pedigrees, see **Fig 1**).

Between patients a, c, and d no striking differences in severity or frequency of the disease could clinically be ascertained.

Individual b2 claimed that blistering was present already at birth and was more generalized in early childhood. With this anamnesis, a K type cannot be certainly excluded.

All analyzed family members were examined carefully by one of us (WK, LBT, BPK).

Mutation detection Genomic DNA was isolated from ethylenediamine tetraacetic acid blood samples. Exons 4–6 of K14 and Exons 1 and 5 of K5 were amplified by polymerase chain reaction using Taq DNA polymerase (Boehringer, Mannheim, Germany). Primer sets were as follows: (i) exons 4–6 of K14, 5′-TGA-CTG-TGG-ACT-GTC-CCT-GGC-TTG-CA-3′ and 5′-TGG-GGG-GGG-CGG-ACT-AAG-GGG-AG-3′. These primers have a high homology to the K14 pseudogene. In order to diminish pseudogene contamination (which in fact can be seen in **Fig 2*a***, C→A in 274) the annealing temperature in polymerase chain reaction was upregulated as high as possible. (ii) Exon 1 of K5, 5′-GCT-GGC-TTT-GGA-GGT-GGC-TTC-GGT-G-3′, and 5′-TGC-AGC-AGG-GTC-CAC-TTT-GTT-TCC-AGA-3′; and (c) exon 5 of K5, 5′-AGA-ACC-AGA-TGA-CCG-ACT-CC-3′ and 5′-GAG-ACA-GTC-ATC-AGA-GCA-CC-3′.

Fifty microliter reactions containing 250 ng DNA, 200 nM of each primer, 200 μM of each dNTP, 10 mM Tris-HCl, 1.25 U Taq DNA polymerase with

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Abbreviations: EBS-WC, epidermolysis bullosa simplex Weber–Cockayne; K, Koebner; K5, keratin 5; K14, keratin 14; KIF, keratin intermediate filament.

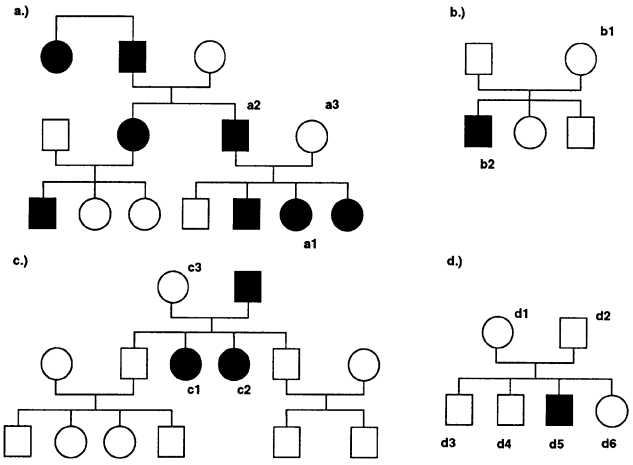

Figure 1. Pedigrees of studied German EBS-WC cases. Analyzed family members are indicated by numbers.

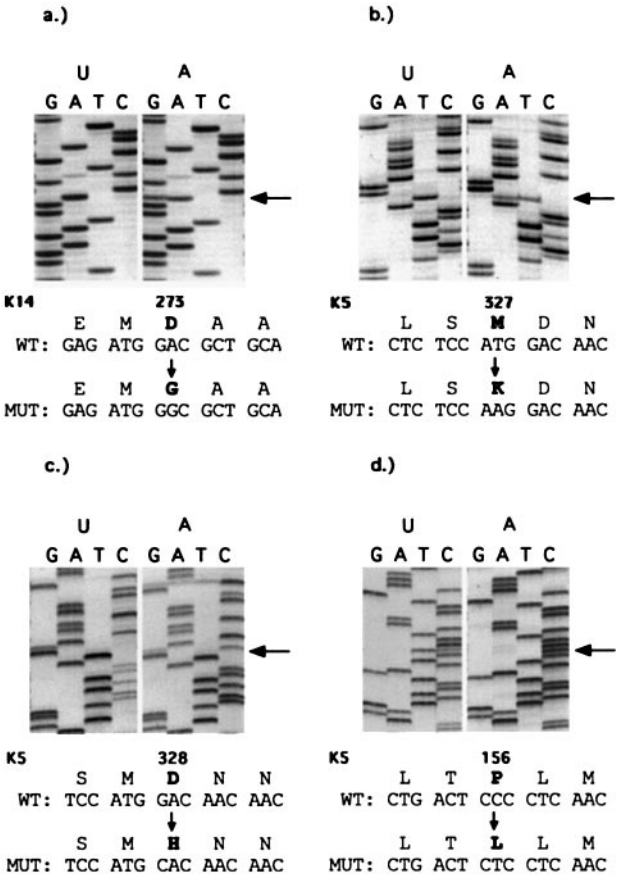

Figure 2. Mutations in K5 and K14. Parts of DNA sequencing gels showing the various detected point mutations in the L12 linker and H1 domain of K5 and K14, respectively. An affected and unaffected family member of each family is shown. *Arrows* indicate the location of the mutation.

optimized $MgCl_2$ and KCl concentrations and pH were initially denaturated at 94°C for 2 min, followed by 35 cycles of 1 min at 94°C, 1 min at 56°C (K5Exon5) or 64°C (K5Exon1,K14Exon4–6) and 1.5 min at 72°C, followed by a 7 min final extension step at 72°C. After treatment with shrimp alkaline phosphatase and exonuclease 1 the polymerase chain reaction products were directly sequenced applying the Sequenase polymerase chain reaction product sequencing kit (USB/Amersham Life Science, Cleveland, OH).

RESULTS

Sequence analyses of K5 and K14 in four German EBS-WC cases detected novel point mutations in the L12 linker-domain of K14

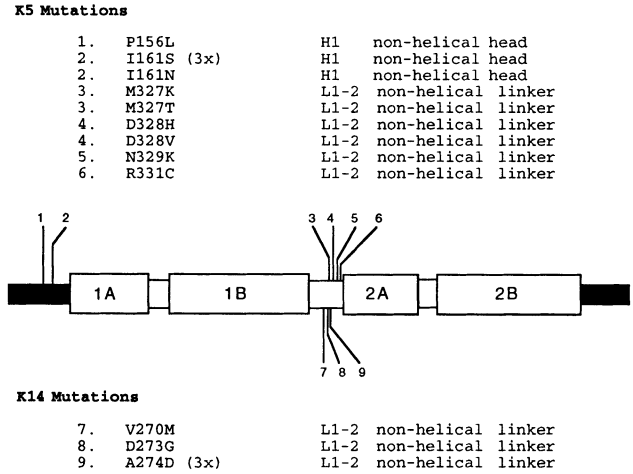

Figure 3. Summary of reported EBS-WC mutations in K5 and K14. Schematical demonstration of the position of reported EBS-WC mutations in the K5 and K14 protein chain. The numbers in parentheses indicate the number of distinct cases if greater than one.

(D273G) (**Fig 2*a***), the H1 domain of K5 (P156L) (**Fig 2*d***), and the L12 linker domain of K5 (M327K and D328H) (**Fig 2*b*, *c***). The novel K14 mutation of patient a1 is an A to G transversion at base 3288 (EMBL accession code: J00124), leading to exchange of an aspartic acid to glycine within the protein. The mutation was also found in the patient's affected father, whereas the clinically unaffected mother did not show the mutation. The novel K5 mutations of patients b2 and c1 altered methionine to lysine at residue 327 (1885T→A) resp. aspartic acid to histidine at residue 328 (1887G→C) (EMBL-AC: M28496). The latter was also found in the patient's affected sister. The mutation found in patient d5 is located in the H1 domain of K5 (1359C→T) at residue 156 and leads to a conversion of a proline to a leucine. None of the mutations occurred in the examined unaffected family members. To discount common polymorphisms we employed restriction enzymes that specifically cut either the mutated or the wild-type sequence. These were HaeII for mutation D273G, NcoI for mutations M327K and D328H (cuts only the wild-type but none of both mutations), and BseRI for mutation P156L. We could exclude the presence of each of the mutation in 50 unrelated controls.

DISCUSSION

The pathogenetical relevance of the here described mutations is supported by two lines of evidence. In all of our and earlier analyzed cases of EBS-WC, there existed a strict correlation between the mild clinical phenotype and the region of the detected mutation in K5 or K14. Furthermore, all these mutations lead to the substitution of highly conserved amino acid residues and are located either nearby or at the same codons as previously described mutations. **Figure 3** demonstrates schematically the clustering of so far reported EBS-WC mutations at three distinct protein structural domains of K5 and K14.

How these defective keratins impair normal KIF stability is not well understood. Indications of how the H1 domain might be involved in the organization of KIF were gained by recent chemical cross-linking studies (Steinert and Parry, 1993; Steinert *et al*, 1993). This model of KIF structure postulates linear arrays of keratin dimers. The end of the 1A domain of one dimer is connected by a 10–11 residue head-to-tail overlap to the 2B end of the next dimer in line. Juxtaposed dimer chains are predicted to be directed anti-parallel with the keratin dimers either aligned staggered or in close axial register. In this model the H1 subdomain could play a critical role for lateral associations between the juxtaposed keratin dimers.

There are also indications that changes in the L12 linker domain may interfere with the packing of protofilaments. In the anti-parallel staggered mode of alignment, the L12 linker comes very close to the head-to-tail overlap region of the neighbored row of dimers and would be therefore in a position to promote appropriate lateral associations.

For over 10 y there have also been suggestions from computer predictions of secondary structure that the L12 linker may form a β-sheet motif in the alternating 4–5 times repeated apolar–polar stretch. In the mode of alignment where dimers are in close axial register this β-sheet structure could form a stable cluster with the sheet motif of the adjacent dimer (Peter Steinert, personal communication). Nevertheless, these predictions of the conformation of the L12 linker are still speculative at this stage and further experiments are needed to understand its function for KIF stability.

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